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Evaluation of the Transfer and Occurrence of Opium Alkaloids in Poppy Seed Teas Using Preconcentrations with µSPEed® Followed by GC-MS Analysis

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Abstract: Intoxication due to the consumption of poppy seed teas has been reported due to their contamination with opium alkaloids (OAs). In this work, an efficient methodology based on microsolid-phase extraction (µSPEed®) followed by gas chromatography–mass spectrometry (GC-MS) has been optimized to quantify five OAs in poppy seed teas. Nine cartridges (C4, C8, C18, silica, APS, PFAs, PS/DVB-RP, PS/DVB-SCX and PS/DVB-SAX), pH levels, cycles and elution solvents were evaluated. The method was validated and applied to study the transfer of OAs by evaluating water temperatures, infusion times and seed amounts. The highest transfer rates (71% for morphine, 96% for thebaine, and 100% for codeine, noscapine and papaverine) were achieved at 90°C, 5 min, with 4 g. These conditions were used to quantify the OAs in four teas prepared from different seeds. A high amount of morphine (1563 µg/L) was found in one tea, indicating that the seeds had a concentration twice the maximum limit, highlighting the need to warn the population of this dangerous practice.

Keywords: food safety; opium alkaloids; µSPEed; GC-MS; poppy seed tea; transfer; occurrence

Citation: Casado-Hidalgo, G.; Perestelo, R.; Morante-Zarcero, S.; Câmara, J.S.; Sierra, I. Evaluation of the Transfer and Occurrence of Opium Alkaloids in Poppy Seed Teas Using Preconcentrations with µSPEed® Followed by GC-MS Analysis. *Chemosensors* **2023**, *11*, 94. https://doi.org/10.3390/chemosensors11020094

Academic Editors: María José Aliaño-González, Irene Domínguez Pérez and Roberto Romero-González

Received: 28 December 2022
Revised: 23 January 2023
Accepted: 24 January 2023
Published: 27 January 2023

1. Introduction

Poppy seed teas have been used for centuries as home remedies to relieve pain, anxiety and stress [1–4]. Although they do not contain opium alkaloids (OAs) themselves, they can be contaminated by the latex of the plant (*Papaver somniferum* L.), which is rich in OAs (e.g., morphine, codeine, thebaine, papaverine and noscapine). This contamination may be due to poor harvesting practices or insect damage. In recent years, numerous studies on poppy seeds have confirmed the presence of OAs, in many cases in considerably high concentrations and highly dispersed due to heterogeneous contamination and the influence of numerous external conditions (e.g., climate, harvesting time and variety, among others) [5–9]. For example, in previous work, widely varying amounts of each of the analytes were determined: 1.5–249.0 mg/kg of morphine, <MQL-45.8 mg/kg of codeine, <MQL-136.2 mg/kg of thebaine, <MQL-27.1 mg/kg of papaverine and <MQL-108.7 mg/kg of noscapine [5]. Until now, this topic has been quite unknown to the general population, and, as there has been no control, constitutes an important food safety issue. The consumption of poppy seed tea has caused false positives in drug tests because, after consumption, OA concentrations have been found in biological samples, as studied in some works, such as blood, urine and serum [10–16]. It may cause adverse health effects, such as nausea and vomiting, drowsiness, respiratory problems, and dependence, especially among the most vulnerable people, as well as more serious cases of intoxication [2,17–22]. Such is the seriousness of the issue that, since 2019, in the United States poppy seeds

have been considered Schedule II controlled substances, such that their commercialization has been made illegal [1]. However, in Europe, it was not until June 2022 that Regulation (EU) 2021/2142 was introduced, setting the maximum morphine equivalent limits (ME = morphine + 0.2 × codeine) in seeds at 20 mg/kg [23]. To gain toxicologically relevant exposure data, it is important to determine the transfer of OAs from seeds to tea, which has not yet been studied. To this end, it is necessary to develop and validate analytical methods that are able to quantify the OAs present in tea infusions.

For this, it is important to consider the crucial role that sample treatment plays in methods of food analysis, from purifying a sample extract to remove possible matrix interferences to preconcentrating to achieve lower detection limits. One of the most commonly used techniques to date is solid-phase extraction (SPE) [24]. However, miniaturized techniques are increasingly being selected as faster, cheaper and more environmentally friendly due to the smaller volumes of organic solvents and lower amounts of sorbents required [25], following the principles of green analytical chemistry (GAC). Many microextraction procedures with different formats and configurations have been developed. Microextraction by packed sorbents (MEPS) is an interesting miniaturization of the SPE technique which requires small amounts of sorbents. However, another configuration that presents some improvements to MEPS is the microsolid-phase extraction (µSPE) technique [26]. This technique has been introduced to the market by the company EPREP, e.g., with µSPEed® (Victoria, Australia), and is based on the use of cartridges containing sorbents with very small particle sizes, smaller than those used in MEPS (≤3 µm versus 50–60 µm), resulting in larger surface areas. In addition, it involves a pressure-operated unidirectional valve (up to 1200 psi) to remove sample flow in one direction only, unlike the MEPS technique, which has two-directional flow potential (up and down) [26,27]. In addition, there are a variety of sorbents for µSPEed® cartridges, such as unmodified and functionalized silica and polymeric materials, which allow the coverage of different retention interactions that may exist between various target analytes [27]. This technique has been used for the extraction of different families of compounds in foods, from bioactive compounds [27,28] to natural toxins [29,30]. However, as far as we know, this is the first time that µSPEed® has been used for quantifying OAs in foods.

One of the most widely used analytical techniques for the analysis of opium alkaloids is gas chromatography coupled with mass spectrometry (GC-MS) and liquid chromatography tandem mass detection (HPLC-MS/MS) [5–31]. Much of the popularity of GC is due to its very high selectivity and resolution, good accuracy and precision, as well as its wide dynamic concentration range and high sensitivity [32]. GC-MS methods are very useful for the identification of individual compounds using standards or specific libraries and their subsequent quantification. However, an alternative approach can be used for qualitative and quantitative determinations, employing GC as a sensor, with the application of a chemometric tool for data treatment [32].

Hence, the aim of this work was to select the most suitable sorbent and evaluate the efficacy of the µSPEed® technique, following this with a GC-MS analysis for the quantification of OA concentrations in poppy seed teas, to propose an efficient and sensitive analytical methodology to determine the transfer of these alkaloids in teas prepared from poppy seeds under different conditions (water temperatures, infusion times and seed amounts), and, in addition, to monitor OA occurrence in home-made teas prepared with different commercially available poppy seeds under the most unfavorable conditions and to evaluate the risk of consuming poppy seed teas.

2. Materials and Methods

2.1. Reagents and Materials

Standards of morphine, codeine and thebaine were received from Alcaliber S.A.U. (Madrid, Spain), and noscapine and papaverine were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Individual stock standard solutions were prepared at 1000 µg/mL in methanol (MeOH). The intermediate mixed standard solution was prepared at 100 µg/mL

in MeOH. The working standard solutions were prepared by diluting the intermediate mixed standard in MeOH. All of these were stored in darkness at −20 °C.

Methanol (MeOH), chloroform ($CHCl_3$) and sodium hydroxide (NaOH) in HPLC grade were purchased from Fischer Scientific (Loughborough, UK). Hydrochloric acid (HCl, 37% *v/v*) was purchased from Panreac (Barcelona, Spain); trifluoroacetic anhydride (TFA) from Sigma-Aldrich (Zwijndrecht, The Netherlands). Ultra-pure deionized water (18.2 MΩ cm quality) was obtained using a Millipore Milli-Q-System (Billerica, MA, USA) and was used for the preparation of aqueous solutions. Nylon syringe filters (0.45 µm) for the filtration of tea infusions were acquired from Scharlau (Barcelona, Spain). The digiVOL® Digital Syringe and the commercial µSPEed® cartridges tested were acquired from EPREP. Nine types of cartridges with different chemical structures—six silica-based: C_4 (tetrylsilane), 3 µm/300 Å; C_8 (octylsilane), 3 µm/120 Å; C_{18} (octadecylsilane), 3 µm/120 Å; APS (WAX, weak anion exchanger), 3 µm/120 Å; PFAs (50% WAX, 50% C_{18}), 3 µm/120 Å; and silica, 3 µm/120 Å); and three polymeric-based: PS/DVB-RP (reversed phase), 3 µm/300 Å; PS/DVB-SCX (strong cation exchanger), 3 µm/non-porous; PS/DVB-SAX (strong anion exchanger), 3 µm/non-porous)—were tested.

2.2. OAs in Poppy Seeds and the Study of Their Transfer to Tea

Four samples of edible seeds (S-1, S-2, S-3 and S-4) were purchased in Spain in the middle of 2021 from supermarkets and herbalists. The labelling for certain of them (S-2) gave recommendations for the preparation of calming and sedating infusions. Detailed information on each of these samples can be found in Table S1.

For the study of the transfer of OAs to tea, S-1 seeds were used that had been previously washed and dried following our previous work [5] and the Recommendation published by the European Union in 2014 to reduce OA contents in seeds [33]. This decision was made because the concentrations of OAs that can be found in the same batch of seeds can be highly disparate due to the heterogeneous contamination that can occur. Once the seeds were washed and free of OAs, they were spiked to the intermediate level of concentration of validation (20 mg/kg), which is the maximum permitted limit legislated [23].

The spiked seeds were subjected to the transfer study, which consisted of studying three factors of infusion (temperature, time and seed amount) at two levels, each with 100 mL of deionized water. The levels of each factor to be studied were selected according to the International Standard ISO 3103 protocol [34] and the instructions of the manufacturers in order to simulate the real conditions in which consumers carry out their preparations. Temperature was studied at two levels, 90 and 100 °C, since in online forums and even on some of the seed labels, infusion at 90 °C was recommended to avoid degradation of the OAs. The times selected were 5 and 10 min, and the amounts studied were 2 and 4 g. Therefore, a matrix of 8 studies was obtained, as shown in Table S2. For this, 2 or 4 g of poppy seeds were weighed in an analytical balance (± 0.1 mg) and infused with 100 mL of deionized water at 90 or 100 °C for 5 or 10 min. Then, the infusion was strained and cooled to room temperature. Later, the sample was filtered through a nylon syringe filter (0.45 µm) before being purified by µSPEed® and analyzed by GC-MS. The areas obtained for each replicate were interpolated on the matrix-matched calibration to obtain the concentration of OAs in the tea. In addition, transfer rates (%) were calculated by comparing the concentration obtained in the tea and the concentration spiked in the seeds. Finally, with the conditions that gave a higher transfer, infusions of the four poppy seeds were made to evaluate the concentration that can be ingested through the infusion and to assess the risk. Three consecutive infusions were prepared with each poppy seed sample by taking three different portions from each sample to obtain the mean of the concentrations with the ± standard deviation (SD).

2.3. µSPEed® Extraction Procedure for OAs in Tea Infusions

The µSPEed® procedure was performed with a digiVOL® Digital Syringe with an automatic syringe of 250 µL at a constant flow rate of 2500 µL/min. Nine available

sorbents (C_4, C_8, C_{18}, PFAs, APS, silica, PS/DVB-RP, PS/DVB-SCX and PS/DVB-SAX) were evaluated and compared to select the ones most efficient with respect to OAs.

First, the µSPEed® parameters were optimized. To achieve this, it was evaluated whether the derivatization of OAs was more appropriate before or after the µSPEed® procedure. Subsequently, the pH of the sample load (pH 3, 7 and 9) was evaluated with all cartridges. The medium selected for loading was water, as it was for the infusions and the sample medium. The loading onto the cartridge can occur in one of two modes: draw–effect (the sample is discarded in the same vial after each extraction cycle, to be sucked in again to ensure complete adsorption) or extract–discard (the sample is discarded in a waste vial after each extraction to ensure preconcentration). First, the draw–effect mode was tested with 3, 5 and 10 cycles to evaluate whether retention increased with increasing cycles. Subsequently, the elution solvent was optimized (MeOH with 10% formic acid, unmodified MeOH, MeOH with 10% NaOH, and $CHCl_3$). Lastly, the number of cycles for extract–discard was optimized (10, 12 and 15 cycles) to ensure the maximum possible preconcentration once the other parameters had been optimized.

Finally, the µSPEed® procedure was carried out using the PS/DVB-RP cartridge previously selected as the most suitable sorbent to extract OAs in the optimization step. Figure 1 shows the µSPEed® procedure under the optimized conditions.

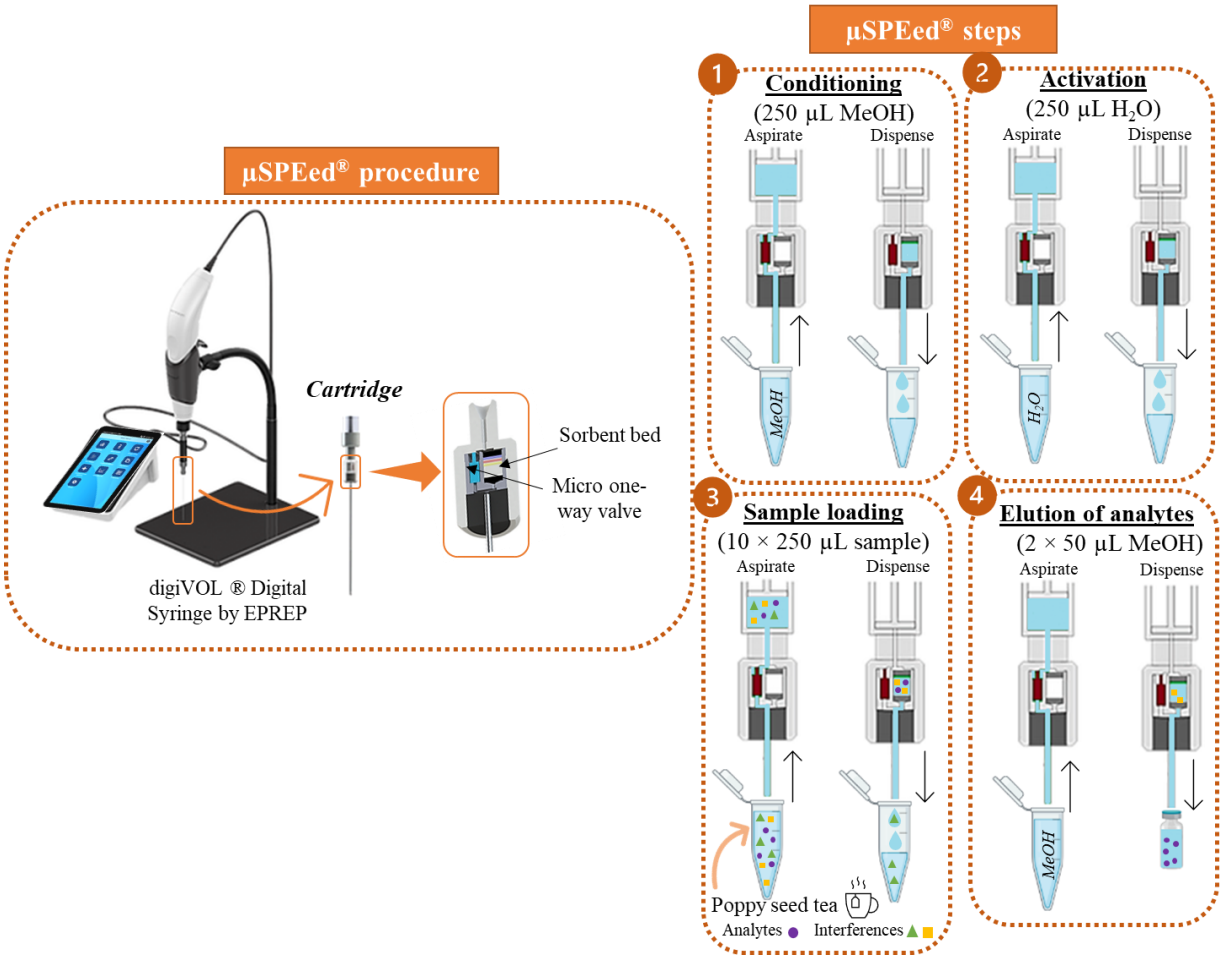

Figure 1. Graphical scheme of the optimized µSPEed® procedure for opium alkaloid extraction using the digiVOL® Digital Syringe (EPREP, Mulgrave, Victoria, Australia).

The first step was the activation of the cartridge with 250 µL of MeOH; later, it was conditioned with 250 µL of water. Finally, the extract–discard mode was chosen and ten different 250 µL aliquots of the filtered infusion were used (250 µL, 10 cycles in extract–

discard mode). No washing step was carried out. In the end, the analytes were eluted into a vial with two different 50 µL aliquots of MeOH (2 × 50 µL). The extract collected in the vial was evaporated and derivatized for subsequent GC-MS analysis. Between each extraction, to ensure proper reuse of the cartridge and to avoid a memory effect (carry-over), and as a conditioning step before the next extraction, the cartridge was washed with two different aliquots of 250 µL of MeOH (2 × 250 µL). Each cartridge was reused more than 100 times.

2.4. Derivatization of OAs and GC-MS Analysis

After the µSPEed® procedure, the extract collected in the vial was evaporated and reconstituted with 100 µL of $CHCl_3$ and 100 µL of TFA at 60 °C for 20 min for derivatization [11]. Finally, the extract derivatized was evaporated and reconstituted in 20 µL of MeOH for GC-MS analysis.

GC-MS analysis was performed using an Agilent 6890 N (Palo Alto, CA, USA) gas chromatograph system coupled to an Agilent 5975 quadrupole Mass Selective Detector. The injection volume was 5 µL, and the mode was splitless mode. The injector temperature was 280 °C, and helium was used as a gas carrier at 1.3 mL/min. For the chromatographic separation, an HP-5 capillary column ((5%-phenyl)-methylpolysiloxane nonpolar phase, 30 m × 320 µm i.d., 0.25 µm film thickness; Hewlett Packard, Palo Alto, USA) was used and was thermostated at 220 °C for 1 min then raised up to 300 °C at 6 °C/min and held for 1 min, resulting in a method of 15.33 min. The ionization was achieved using an electron ionization source (EI) at 70 eV. The transfer line, ion source and quadrupole analyzer temperatures were maintained at 220, 180 and 200 °C, respectively, and a solvent delay of 4 min was selected.

Initially, a full-scan (FS) MS acquisition mode with a mass range of fragment ions from 50 to 500 amu was employed for qualitative detection, to select the characteristic fragment ions for the target OAs. For this purpose, a standard solution of the analytes at a concentration of 50 µg/mL, previously derivatized with the optimized protocol, was used. The selected ion monitoring (SIM) mode was used as a quantitative scan, whereby the molecular ion of the analyte was monitored in a narrow amu window. For each compound the most abundant fragment ions were used (Table 1). In this MS acquisition mode, only the selected fragment ions with m/z values of interest, rather than a wide range of m/z values, are collected, significantly improving the selectivity and consequently the detection limits of the technique.

Table 1. Retention time and m/z values of fragment ions selected for the quantification and confirmation of the five opium alkaloids.

Compound Name	Quantification 1 (m/z)	Ion 2 (m/z)	Ion 3 (m/z)
Codeine	299	229	162
Morphine	285	215	162
Thebaine	311	296	242
Papaverine	338	324	154
Noscapine	220	215	205

2.5. Analytical Method Validation

There is no official regulation regarding analytical performance requirements for quantifying OAs in food or other matrices. Therefore, the validation of the method was performed following the criteria described in SANTE/11312/2021 for pesticides, in EC Regulation No. 401/2006 and in the ICH Q_2(R1) guidelines [35–37]. The validation method was performed for linear dynamic range (LDR), matrix effect (ME), method detection and quantification limits (MDL, MQL), accuracy, precision and selectivity. The validation was carried out with a spiked sample previously washed and dried (S-1).

Accordingly, LDR was evaluated at six concentration levels with standard solutions prepared and analyzed using the proposed µSPEed® procedure. Concentration ranges were selected according to the sensitivity of the GC-MS system to each target analyte, as

well as the amounts expected in the tea infusions. Matrix-match calibration lines were prepared in the same way, but instead of using standard solutions, the tea obtained from the poppy seeds (S-1) that had been previously washed and dried was spiked. All calibration curves were obtained with the mean peak area of each analyte versus analyte concentration and fitted by least-squares linear regression. Matrix effects were determined by comparing the slopes of the calibration equations obtained from matrix-matched and solvent-based calibration curves for each analyte, calculated using the following formula: (slope matrix-matched/slope solvent-based -1) $\times$ 100. The ME is negligible when it is lower than $+/-20\%$ [35], representing signal enhancement when ME values are greater than 20% and signal suppression when values are greater than -20%. The sensitivity of the method for each sample was determined according to the MDLs and MQLs of the OAs from the analysis of the lowest concentration analyzed (5 µg/L), which yielded signal-to-noise (S/N) ratios of 3 and 10 (when the quantification ion was monitored), respectively.

The accuracy, expressed as recovery percentage (%), was assessed by comparing the areas obtained for spiked samples (n = 6) with known concentrations of analytes and by performing the µSPEed® procedure with those areas obtained for simulated samples (samples spiked at the same concentration but at the end of the µSPEed® procedure, prior to the derivatization step for GC-MS analysis). The recovery assays were performed by spiking the samples at three concentration levels: 800 µg/L (high level, HL), 400 µg/L (medium level, ML) and 100 µg/L (low level, LL). In the absence of legislation setting a maximum limit for OAs in poppy seed tea infusions, the Regulation (EU) 2021/2142 maximum limit of 20 mg/kg in seeds was followed, and the concentrations of OAs in the teas were estimated assuming complete transfer. Thus, for the seeds with 20 mg/kg of OAs, the concentration in tea (2 g of seed in 100 mL of water) was 400 µg/L, this being the intermediate level of validation. The recovery values should be between 70 and 120%. On the other hand, method precision was evaluated in terms of repeatability (intra-day) and reproducibility (inter-day), using the same validation levels as for the accuracy assays. For repeatability (expressed as a percentage of the relative standard deviation, RSD %), six replicates (n = 6) of the whole procedure were performed on the same day. The reproducibility (also expressed as RSD %) was calculated by analysis of three replicates of a sample, which analyses were carried out in triplicate on three different days (n = 9). According to the validation guidelines, the RSD values for these precision parameters should be $\leq$20%. Selectivity was assessed by the absence of interfering chromatographic peaks at the retention time for the target analytes.

2.6. Statistical Analysis

Statistical analyses were performed using the SPSS 25.0 statistical package (SPSS Inc., Chicago, IL, USA) by one-factor ANOVA analysis. Differences were considered significant for p-values $\leq$ 0.05. For the Pareto charts, Statgraphics Centurion software (version 19.3.03) was used to show the significant effects of each of the variables and their respective interactions.

3. Results and Discussions

3.1. OA Derivatization Step and GC-MS Analysis

First, derivatization was performed following the protocol of Hayes and Krasselt (1987) [11], using 100 µL of $CHCl_3$ and 100 µL of TFA for 20 min at 60 °C. A quantity of 1 mL of a 50 µg/mL standard solution was injected into the GC-MS equipment, and the MS acquisition was carried out in FS mode with a mass-to-charge ratio ranging from 50 to 500 (m/z) to identify each OA based on its mass spectrum. Considering the low levels at which OAs can occur, the full-scan MS detection is not the most suitable approach for this purpose. The low sensitivity and selectivity of this MS acquisition mode were overcome using the SIM mode, in which the most abundant OA fragment ions seen in the full scan were selected for quantitative purposes (Table 1). This MS acquisition mode presents high selectivity for every single OA in the sample as well as high sensitivity and allows

the confirmation of analyte identity and accurate quantification of analytes. This allows the achievement of lower detection and quantification limits by improving the selectivity and sensitivity of the instrumental analysis. A chromatographic method with a starting temperature of 80 °C and rising to 300 °C with a ramp of 4 °C/min, corresponding to an analysis time of 45 min, was used. Thus, it was possible to separate and identify each of the analytes with their respective ions to create the SIM method that allows increasing specificity and the elimination of interference.

Once the SIM method was created, the analysis time was reduced by modifying the oven temperatures. For this, the first step was to increase the starting temperature from 80 °C to 180 °C, since the retention time of the first analyte was 24.14 min. This decreased the retention time of the first analyte to 12.10 min and the analysis time to 31 min. Then, to shorten it further, the starting temperature was raised to 200 °C, achieving a codeine retention time of 7.30 min and a total time of 21 min. Finally, the starting temperature was raised to 220 °C and the ramp was made faster—instead of 4 °C/min, it was set to 6 °C/min; thus, the retention times for each of the analytes were: codeine, 5.06 min; morphine, 5.46 min; thebaine, 6.31 min; papaverine, 9.44 min; and noscapine, 12.77 min, with a total time of 14.33 min, as shown in Figure 2. In addition, 1 min of hold time at the end of the method was increased to ensure the cleanliness of the column between injections. The spectra of each compound can be used for the rapid identification of compounds before quantification, i.e., from the TIC (Figure 2) they can be extracted and quickly checked and identified.

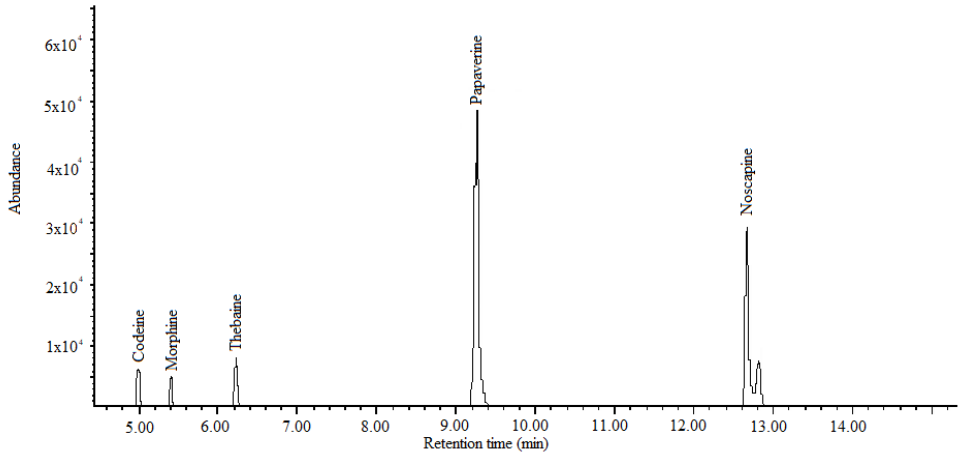

Figure 2. Total ion chromatography (TIC) obtained for each opium alkaloid using the SIM mode for a poppy seed tea extract spiked at a low level of validation (100 µg/L).

Once the chromatographic method was optimized, some tests were carried out to optimize the derivatization step. For this, different derivatization solvents were tested ($CHCl_3$, MeOH, ethyl acetate and hexane) at different temperatures (40 and 60 °C) and times (10, 20 and 40 min). Finally, the optimum conditions selected were: $CHCl_3$ at 60 °C for 20 min, thus confirming that the protocol used in the work of Hayes and Krasselt (1987) [11] to derivatize morphine and codeine was also the most suitable for the derivatization of all OAs.3.2. µSPEed® Procedure Optimization.

To optimize the µSPEed® procedure, nine cartridges were tested due to the different chemical properties of the OAs, papaverine and noscapine having the lowest water solubilities (0.013 and 0.18 g/L, respectively) and morphine having the highest (10.20 g/L) [31].

First, nine cartridges were evaluated by performing the derivatization step before and after to see when it was more efficient to perform it. For this, the cartridge was activated and conditioned with 250 µL of MeOH and H_2O, respectively, and the loading step was performed with 250 µL of a 5 mg/L standard solution in water in a 3-fold draw–effect cycle. The first studies were performed at a higher concentration for the target analytes to be detected by GC-MS. Then, the analytes were eluted with 50 µL of MeOH twice (50 µL × 2).

As shown in Figure 3a, the areas obtained were generally larger when derivatization was performed after the µSPEed® procedure. Thus, it was decided to perform derivatization after the µSPEed® procedure.

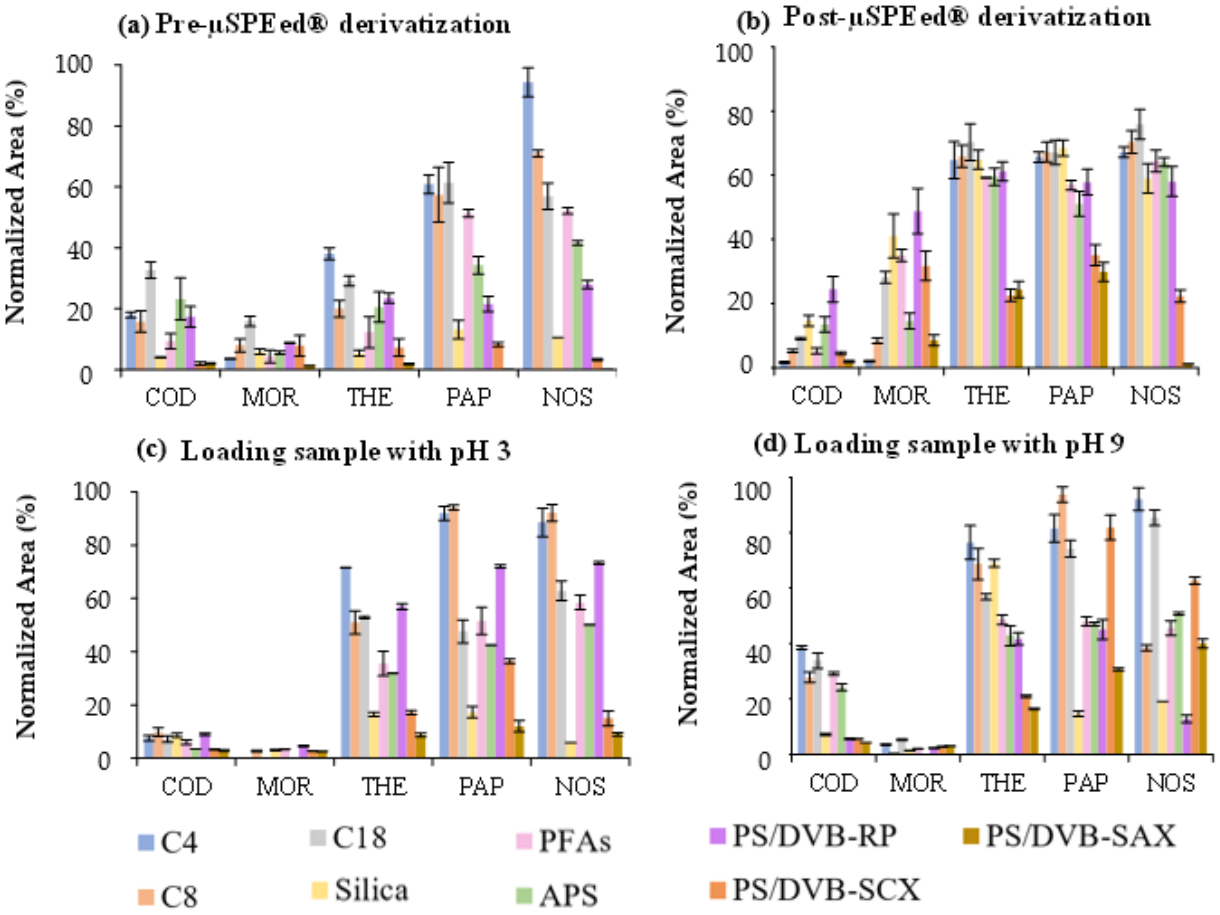

Figure 3. Normalized areas (%) obtained for OAs in the optimization of the µSPEed® procedure with nine cartridges: derivatization before (**a**) and after (**b**), acidifying to pH 3 (**c**) or basifying to pH 9 (**d**) in the loading step. All assays were performed with 5 mg/L standard solutions in triplicate; error bars indicate the relative standard deviations (RSDs %).

Subsequently, as no cartridge was shown to be clearly better than the others for all the analytes, due to the different chemical properties of OAs, in addition to testing all the cartridges by performing the loading step at pH 7, they were tested by acidifying the 5 mg/L standard solution with HCl (pH 3) and basifying it with NaOH (pH 9). Comparing the areas obtained at pH 7 (Figure 3b) and those obtained at pH 3 (Figure 3c) and 9 (Figure 3d), the structural and polarity differences between the analytes were even more evident. Some cartridges showed larger areas with lower pHs, such as C_4, and others showed higher pHs, such as C_8 or PS/DVB-SAX. However, the areas obtained with pH 3 and 9 for morphine were very small for all cartridges. Therefore, pH 7 was selected, and, finally, it was decided to select the silica and PS/DVB-RP cartridges, which showed the best responses for morphine and codeine, and for the rest of the analytes there was little difference between the cartridges with respect to the size of the areas.

Afterwards, the two selected cartridges were studied by increasing the draw–effect cycles in the loading step to obtain the maximum possible adsorption of 5 mg/L of standard solution and to be able to select the cartridge that could adsorb the most. As shown in Figure 4a, after comparing the areas obtained, it was seen that the PS/DVB-RP cartridge, after 10 cycles, achieved a considerably larger area.

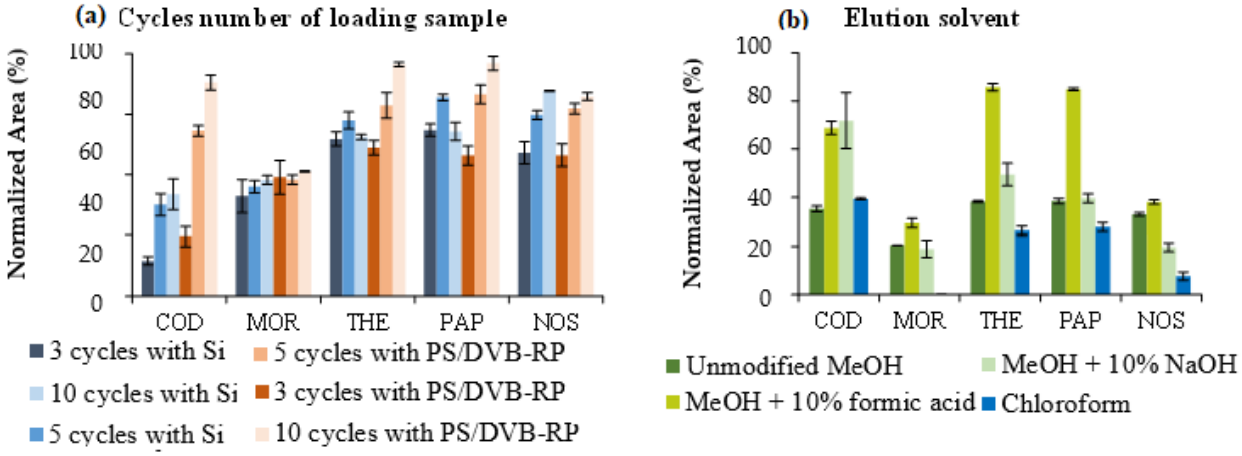

Figure 4. Normalized areas (%) obtained with the silica and PS/DVB-RP cartridge with 3, 5 and 10 draw–effect cycles in the loading step at pH 7 (**a**) and with the PS/DVB-RP cartridge with a different elution solvent with a pH of 7 in the loading step (**b**). All assays were performed with 5 mg/L standard solutions in triplicate; error bars indicate the relative standard deviations (RSDs %).

Later, different elution solvents (MeOH unmodified, MeOH with 10% formic acid, MeOH with 10% NaOH, and $CHCl_3$) were evaluated with the other optimal conditions, with 5 mg/L of standard solution in the loading step. As shown in Figure 4b, MeOH with 10% formic acid resulted in the largest area shown for all analytes.

Finally, as one of the advantages of the µSPEed® technique is the possibility of preconcentrating sample extracts to obtain lower instrumental limits, the studies were carried out with the optimal conditions, but with 10, 12 and 15 extract–discard cycles in the loading step. For this purpose, this study was carried out at the low validation level (100 µg/L) to determine whether a preconcentration could be carried out with the µSPEed® to allow the desired concentration levels to be quantified in the equipment. The recovery values were calculated with their respective simulated values. The values obtained by performing 10 cycles were 91 ± 8% for morphine and around 100% recovery for the rest of the OAs; performing 12 cycles, codeine, thebaine and papaverine showed around 100% recovery, but morphine showed 37 ± 12% and noscapine 85 ± 9%; and, performing 15 cycles, morphine and noscapine showed close to 15% recovery, codeine 40 ± 9%, papaverine 65 ± 9% and thebaine 82 ± 9%. For this reason, the µSPEed® procedure with 10 cycles was selected, which allowed a preconcentration factor of 10 and the obtainment of adequate recovery values.

3.2. Validation of the Proposed Method Based on µSPEed® Followed by GC-MS

The results of the validation of the proposed method based on µSPEed® followed by GC-MS for the quantification of OAs in poppy seed teas are shown in Table 2. The regression lines were obtained by least-squares linear regression analysis of the data and provided excellent correlation coefficient (R^2) values between 0.998 and 1.000 for all analytes.

Table 2. Validation parameters for the proposed method based on µSPEed® followed by GC-MS for the quantification of opium alkaloids in poppy seed teas.

Analytes	Linear Range (µg/L) [a]	Matrix-Matched Calibration (R^2)	MDL (µg/L) [b]	MQL (µg/L) [c]	ME [d]	Accuracy [e]		Precision (%RSD) [e]	
						Recovery (% ± SD)		Intra-Day	Inter-Day
Codeine	5–800	$y = 3.7 \times 10^6 \times - 4.1 \times 10^2$ (0.999)	0.3	1	8	LL	91 ± 8	9	11
						ML	101 ± 4	4	7
						HL	99 ± 1	1	4
Morphine	5–800	$y = 3.0 \times 10^6 \times - 1.6 \times 10^4$ (0.998)	0.5	1.6	−4	LL	90± 8	8	10
						ML	102 ± 7	7	13
						HL	90 ± 3	3	7

Table 2. *Cont.*

Analytes	Linear Range (μg/L) [a]	Matrix-Matched Calibration (R^2)	MDL (μg/L) [b]	MQL (μg/L) [c]	ME [d]	Accuracy [e]		Precision (%RSD) [e]	
						Recovery (% ± SD)		Intra-Day	Inter-Day
Thebaine	5–800	$y = 3.6 \times 10^6 \times + 9.6 \times 10^3$ (0.999)	0.2	0.7	1	LL	91 ± 4	5	9
						ML	100 ± 10	10	14
						HL	101 ± 3	3	4
Papaverine	5–800	$y = 2.9 \times 10^7 \times - 2.3 \times 10^5$ (1.000)	0.06	0.2	−6	LL	89 ± 4	4	7
						ML	100 ± 7	7	10
						HL	95 ± 6	6	10
Noscapine	5–800	$y = 1.6 \times 10^7 \times - 3.3 \times 10^5$ (0.999)	0.07	0.2	10	LL	93 ± 8	9	13
						ML	102 ± 7	7	10
						HL	108 ± 7	6	11

[a] The linear range is expressed in μg/L of poppy seed tea; [b] MDL: method detection limit is in μg/L of poppy seed tea; [c] MQL: method quantification limit is in μg/L of poppy seed tea; [d] ME (%): matrix effect (dividing the purified matrix slope by the solvent slope − 1) × 100; [e] Accuracy and precision were obtained by spiking samples at three concentration levels: low level (LL, 100 μg/L), medium level (ML, 400 μg/L) and high level (HL, 800 μg/L).

The method showed low MDL and MQL values expressed in μg/L of poppy seed tea (Table 2): 0.06 and 0.2 μg/L for papaverine, 0.07 and 0.2 μg/L for noscapine, 0.2 and 1 μg/L for thebaine, 0.3 and 1 μg/L for codeine, and 0.5 and 1.6 μg/L for morphine, respectively.

The MEs were calculated by comparing the slopes of the matrix and solvent calibration curves. As shown in Table 2, the MEs of the proposed methodology were negligible, as all values were within +/−20%. This means that the μSPEed® procedure was able to eliminate all possible matrix effects. For this reason, solvent regression lines could be used to quantify the samples, simplifying the analysis [35].

Accuracy and precision were evaluated at three different concentration levels: 100 μg/L (LL), 400 μg/L (ML) and 800 μg/L (HL). Accuracy was expressed as the average recovery obtained comparing six samples ($n = 6$) spiked with the corresponding values with their simulated samples. As shown in Table 2, recovery values were adequate according to the guidelines [35] for all validation levels (between 89 and 108%). In addition, satisfactory results were obtained for intra-day and inter-day precision at the three concentration levels, since the RSD values were lower than 20%, according to the guidelines [35], and the lowest value was 14% (Table 2). Furthermore, as shown in Figure 2, good selectivity for the method was demonstrated, since no interfering peaks were found at the retention time for the target analytes.

The most significant advantages of the proposed method over previously published ones were that this method, in addition to extending to morphine, codeine and thebaine, also extends to papaverine and noscapine, which can be even more toxic [17]. In addition, by performing the μSPEed® procedure, it was possible to preconcentrate the extract 10 times more, such that the present method has 10-fold lower limits than those of the work of Li et al. (2021), which also used GC-MS to quantify poppy seed teas, their obtained MDL and MQL values being 2.5 and 10 μg/L, respectively, for morphine and codeine and 20 and 50 μg/L for thebaine [1]. On the other hand, the present method showed a wide linear response range, allowing us to quantify infusions made with seeds contaminated with amounts of OAs (40 mg/kg) twice those of the legislated maximum limit (20 mg/kg), as quantified in numerous studies [5–7]. However, the method developed by Powers et al. (2019), which used HPLC-MS/MS, only reached 500 μg/mL, i.e., 25 mg/kg in seeds, with a 100% transfer rate [3]. So, different dilutions are needed to quantify samples when the concentrations are outside the calibration range.

Overall, the proposed method based on μSPEed® followed by GC-MS analysis proved to be an efficient strategy for the extraction and quantification of five OAs in poppy seed teas, showing excellent performance in terms of linearity, sensitivity, matrix effects, precision and accuracy.

3.3. Transfer Study of OAs from Poppy Seeds to Tea Infusions

To determine the level of transfer of OAs from poppy seeds to tea infusions, three factors (water temperature, infusion time and seed amount) were studied at two levels each (n = 8), each condition being studied in triplicate. For this, the seeds (S-1) were spiked to the maximum legislated limit (20 mg/kg) [23], having previously been washed and dried. Each of the studies was performed with 100 mL of deionized water, and, after cooling to room temperature (25 ± 1 ºC), the preparations were filtered through a nylon syringe filter; finally, the proposed μSPEed® procedure was applied, followed by the GC-MS analysis.

The areas obtained were interpolated on the respective matrix-matched calibrations to calculate the concentrations of OAs in the teas. The concentrations obtained were compared, as shown in Figure 5a. ANOVA tests were performed to determine the statistically significant differences between each of the studies performed, and the transfer ratios (%) were calculated to determine the conditions under which the highest concentrations of OAs could be obtained in the teas. By spiking the seeds with the maximum limit established in the legislation and using two quantities of seeds (2 and 4 g), the concentrations that could be obtained in these teas were 0.4 and 0.8 mg/L, respectively, if 100% transfer occurred. As can be seen in Figure 5a, with 4 g of seeds at 90 °C for 5 min, statistically significantly higher concentrations of codeine, morphine and thebaine in teas were obtained.

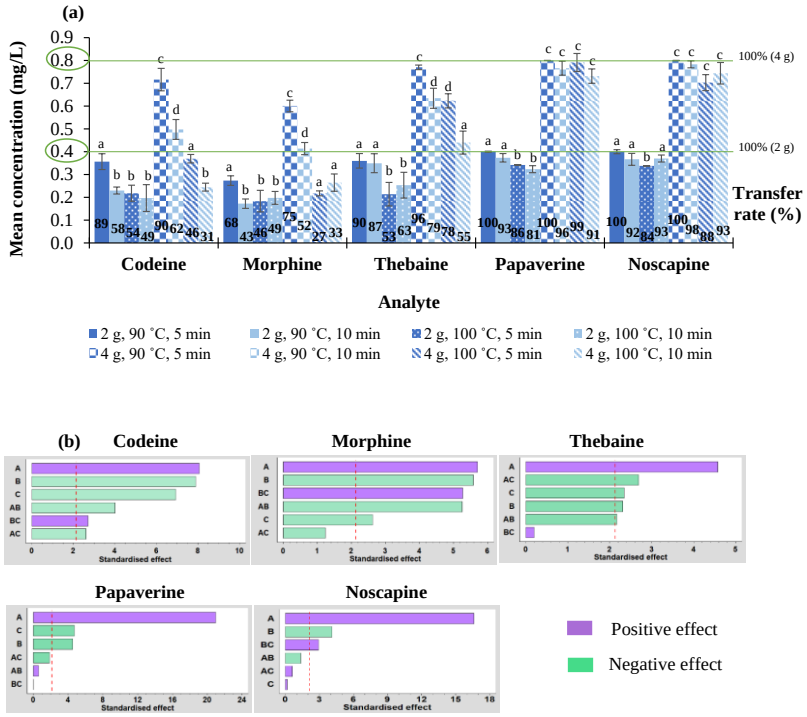

Figure 5. Mean concentrations (n = 3) obtained in each transfer study with their transfer rates (%). The same letters mean that there were no statistically significant differences; different letters mean that there were such differences ($p \leq 0.05$) (**a**). Pareto Chart of the standardized effect of each of the responses (concentration of each analyte), showing the three factors: (A) poppy seed amount (g), (B) infusion time (min) and (C) temperature (°C) (**b**).

This was because, the greater the amount of seeds, the greater the concentration of OAs obtained, i.e., 100 mL of water was still sufficient for the extraction of all opiates from the seeds. Therefore, transfer ratios (%) lower than 100% should not be due to a lower transfer but rather to thermal degradation, as confirmed by some published works [7,33,38,39]. This degradation is mainly observed in codeine and morphine, which are the most thermally sensitive analytes [38]. Thus, between the results of the studies performed at 90 °C and those performed at 100 °C, statistically significant differences were

observed for all analytes, although they were much lower in the cases of papaverine and noscapine, in which thermal degradation was affected less. Regarding infusion time, higher concentrations could be expected with longer times, as the contact time between water and seeds was increased. However, it was observed that increasing the time could in some cases decrease the concentration of OAs in the tea. This may have been due to increased degradation with increasing exposure time of the OAs at high temperatures. All of this is confirmed by the Pareto charts shown in Figure 5b, in which effect A (amount of seeds) has a very significant positive effect on all analytes, effect B (infusion time) has a negative effect on all analytes (the effects being much higher in the cases of codeine and morphine) and effect C (temperature) has a negative effect on all analytes except noscapine, for which no statistically significant difference was observed.

Therefore, the conditions selected for the highest rate of transfer or lowest rate of degradation were 4 g at 90 °C for 5 min, obtaining 90% transfer in codeine, 75% in morphine, 96% in thebaine, and 100% in papaverine and noscapine.

3.4. Occurrence of OAs in Tea Infusions from Different Poppy Seeds

To determine the occurrence of OAs in poppy seed teas prepared using different samples of poppy seeds, the conditions with greater rates of transfer were used. For this reason, 4 g of each of the poppy seed samples were infused with 100 mL of water at 90 °C for 5 min. The studies were performed in triplicate, taking a fresh 4 g of each sample for each infusion to determine the extent of the variation for each seed sample. After cooling to room temperature, the preparations were filtered through a nylon syringe filter, and, finally, the μSPEed® procedure was applied, followed by the proposed GC-MS analysis. The areas obtained were interpolated on the respective matrix-matched calibrations to calculate the concentrations of OAs obtained for each of the teas.

As shown in Table 3, all OAs were quantified for all the poppy seed teas analyzed. Furthermore, as can be seen, the calculated standard deviations of the three replicates were in some cases high. This may have been due to the heterogeneous contamination that seeds suffer, external contamination depending on multiple factors, such as climate, time of harvest, and variety, among others [5,6,8].

Table 3. Occurrence of opium alkaloids (main concentration (μg/L) of three replicates ± standard deviation) in poppy seed teas prepared with four different samples of poppy seeds (S-1, S-2, S-3 and S-4).

Code Sample	Morphine	Codeine	Thebaine	Papaverine	Noscapine	ME [a]
S-1 tea	25 ± 3	4 ± 1	< MQL	8.13 ± 0.02	20.6 ± 0.2	26
S-2 tea	43 ± 25	1.8 ± 0.4	< MQL	8.2 ± 0.2	23 ± 2	43
S-3 tea	227 ± 39	58 ± 42	56 ± 36	11 ± 6	29 ± 6	239
S-4 tea	1563 ± 33	254 ± 35	71 ± 13	29 ± 2	56 ± 1	1614

[a] ME: morphine equivalents = morphine + 0.2 × codeine; <MQL: below the method quantification limit.

Of the four poppy seed teas analyzed, S-1 and S-2 showed the lowest OA contents, morphine and noscapine being the analytes with the highest concentrations in both teas (43 μg/L and 23 μg/L, respectively) and thebaine the one with the lowest (below the MQL). These samples were labelled *Papaver rhoeas* L. (Table S1), which does not contain OAs in its latex, such that its seeds cannot be contaminated. Therefore, the determinations of OAs in these infusions confirmed the mislabeling of the products, as observed in previous studies [5]. S-3 showed a higher content of all OAs, especially morphine 227 μg/L, and S-4 was the sample with the highest concentration of all OAs, giving up to 1563 μg/L of morphine, 254 μg/L of codeine, 71 μg/L of thebaine, 56 μg/L of noscapine and 29 μg/L of papaverine. This sample was diluted 1:2 to quantify it within the calibration line and then a recalculation was performed to give the final concentration. Considering the transfer rates of 75% for morphine and 90% for codeine, the estimated quantity for S-4 poppy seeds expressed in morphine equivalents is 54 mg/kg, which is higher than the legislated

limit (20 mg/kg). It should be noted that the seeds were purchased in 2021 and therefore could not have been expected to comply with current legislation, according to which, if the seeds were marketed before 1 July 2022, they can remain in trade until the best-before date is reached [23]. In addition, the acute dose of morphine equivalents set by the EFSA in 2018 is 10 µg per kg body weight. So, for a 20 kg child, the acute dose would be 200 µg, and for a 60 kg adult it would be 600 µg morphine equivalents. Following the consumer recommendations of some manufacturers of 3 cups of tea per day, if the seeds used for the infusion were S-4, 648 µg morphine equivalents would be ingested. Therefore, the consumption of tea made from these seeds poses a health risk for both adults and children. Furthermore, it should be noted that the legislation only applies to morphine and codeine, and, as observed for all teas, considerable amounts of thebaine, papaverine and noscapine are also present, which can be even more toxic, as claimed by the health authorities [17]. This highlights the need to control these analytes and the importance of developing analytical methods to analyze them in order to develop adequate legislation accordingly.

Results from other works that quantified OAs in home-brewed poppy seed teas also showed considerably high amounts of OAs in the infusions. For example, in the work of Li et al. (2021), the total mean concentrations of morphine, codeine and thebaine estimated for poppy seeds, from the analysis of three repeated infusions of 2 g of seeds with 6 mL of water acidified with 5% lemon juice (heated at 90 °C for 10 min), ranged from 1.1 to 1926, 20.2 to 311, and 9.0 to 100 mg/kg, respectively. These authors indicated that most OAs were extracted in the first brew (around 80% of the total opiate yield), so potential overdose could occur with some tea samples when large quantities of seeds are used in brewing [1]. In the work of Powers et al. (2019), different teas were prepared (i.e., 85 g of seeds in 150 mL, 6 g of seeds in 30 mL and 35 g of seed powder in 100 mL with 5% lemon juice in water, for 10 min at 23 °C and 94 °C) and the concentrations of morphine, codeine and thebaine estimated in the seeds from the poppy seed teas were <1–2788 mg/kg, <1–247.6 mg/kg and <1–124 mg/kg, respectively [3]. In the work of Montgomery et al. (2019), the average concentrations of morphine (mg/kg of seed) determined from the beverage extractions were found to be between 155 and 223 mg/kg. In this work, four poppy seed tea samples were prepared utilizing extractants commonly described in online drug forums (i.e., lemonade, lemon-flavored iced tea, and 10% concentrated lemon juice and water) and compared with a control solution containing acetic acid (1 g of seed in 5 mL). These results showed that, from all the beverages, significant amounts of morphine could be extracted from the surfaces of poppy seeds, despite the extractions being carried out at room temperature for 2 h [4].

In short, the consumption of poppy seed tea can potentially be dangerous, especially since consumers can prepare tea with any amount of seeds and do not know the concentrations of OAs they may contain. In addition, as demonstrated in the present study, despite some thermal degradation, morphine and codeine have high transfer rates, as do the rest of the OAs. Therefore, the study of these types of samples is essential in order to highlight the danger of this consumption practice and to warn the authorities of the need to control it, as it may pose a potential risk to the health of consumers. Even if the maximum amount is currently set at 20 mg/kg and no new seed samples with higher levels can be marketed from 1 July onwards, the authorities should instruct the manufacturers of these products to establish recommendations as to the methods of preparation and the maximum daily amounts to be consumed and provide indications that adverse health effects may occur if these recommendations are not followed.

4. Conclusions

A simple and efficient method was developed for the quantification of morphine, codeine, thebaine, papaverine and noscapine concentrations in poppy seed teas using µSPEed® followed by GC-MS analysis. Of the nine µSPEed® cartridges evaluated, PS/DVB-RP was the one most efficient for OAs and allowed, with optimized conditions, the elimina-

tion of possible matrix effects of the extract and 10-times concentrations, thus decreasing the MDLs and MQLs. Once the method was successfully validated, it was applied to study the rate of transfer of opiates from poppy seeds to teas. For this, the influence of three factors (temperature, time and quantity) was evaluated at two levels and it was determined that 4 g of seeds at 90 °C for 5 min were the conditions that produced the highest rate of transfer or less thermal degradation (75% morphine, 90% codeine, 96% thebaine, and 100% papaverine and noscapine). With these conditions, four teas made with different poppy seeds were quantified, and for all of them all the OAs were determined, one of which showed high morphine (1563 µg/L) and codeine (254 µg/L) contents, indicating that the seeds used contained 53 mg/kg morphine equivalents—twice the maximum limit legislated by the EU (20 mg/kg). Therefore, it is necessary to warn the population of the danger of not following the consumption recommendations, since it may be thought that the OA contents in the seeds are not very high.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors11020094/s1, Table S1: Registration of the different poppy seeds used to make the tea infusions, with descriptions, species and origins; Table S2: Summary of the transfer studies performed with the three factors under study and the two levels for each.

Author Contributions: Conceptualization, I.S., J.S.C. and S.M.-Z.; methodology, G.C.-H. and R.P.; validation, G.C.-H., R.P. and S.M.-Z.; formal analysis, G.C.-H. and R.P.; investigation, I.S., J.S.C., R.P. and S.M.-Z.; resources, G.C.-H.; data curation, G.C.-H.; writing—original draft preparation, I.S., S.M.-Z. and G.C.-H.; writing—review and editing, I.S., J.S.C., S.M.-Z., R.P. and G.C.-H.; supervision, I.S., J.S.C., R.P. and S.M.-Z.; project administration, I.S. and J.S.C.; funding acquisition, J.S.C. and I.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by MCIU/AEI/FEDER, UE for project RTI2018-094558-B-I00. This work was also funded by FCT-Fundação para a Ciência e a Tecnologia through the CQM Base Fund—UIDB/00674/2020—and the Programmatic Fund—UIDP/00674/2020—and by ARDITI-Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação, through the project M1420-01-0145-FEDER-000005—Centro de Química da Madeira—CQM+ (Madeira 14-20 Program). The authors also acknowledge financial support from the Fundação para a Ciência e Tecnologia and Madeira 14-2020 program to the Portuguese Mass Spectrometry Network through the PROEQUIPRAM program, M14-20 M1420-01-0145-FEDER-000008.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Gema Casado-Hidalgo would like to thank the Rey Juan Carlos University for providing her with a mobility grant to carry out a pre-doctoral stay at CQM—Madeira Chemistry Centre.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Li, S.Y.; Swortwood, M.J.; Chi, J.; Yu, C. Determination of morphine, codeine, and thebaine concentrations from poppy seed tea using magnetic carbon nanotubes facilitated dispersive micro-solid phase extraction and GC-MS analysis. *Forensic Sci. Int.* **2021**, *329*, 111052. [CrossRef] [PubMed]
2. Haber, I.; Pergolizzi, J.; LeQuang, J.A. Poppy Seed Tea: A Short Review and Case Study. *Pain Ther.* **2019**, *8*, 151–155. [CrossRef] [PubMed]
3. Powers, F.; Erickson, S.; Swortwood, M.J. Quantification of Morphine, Codeine, and Thebaine in Home-Brewed Poppy Seed Tea by LC-MS/MS. *J. Forensic Sci.* **2018**, *63*, 1229–1235. [CrossRef]
4. Montgomery, M.T.; Conlan, X.A.; Barnett, N.W.; Theakstone, A.G.; Quayle, K.; and Smith, Z.M. Determination of morphine in culinary poppy seed tea extractions using high performance liquid chromatography with chemiluminescence detection. *Aust. J. Forensic Sci.* **2019**, *51*, S225–S228. [CrossRef]
5. Casado-Hidalgo, G.; Pérez-Quintanilla, D.; Morante-Zarcero, S.; Sierra, I. Mesostructured Silica-Coated Magnetic Nanoparticles to Extract Six Opium Alkaloids in Poppy Seeds Prior to Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry Analysis. *Foods* **2021**, *10*, 1587. [CrossRef] [PubMed]

6. López, P.; Pereboom-de Fauw, D.P.; Mulder, P.P.; Spanjer, M.; de Stoppelaar, J.; Mol, H.; de Nijs, M. Straightforward analytical method to determine opium alkaloids in poppy seeds and bakery products. *Food Chem.* **2018**, *242*, 443–450. [CrossRef] [PubMed]
7. Sproll, C.; Perz, R.C.; Lachenmeier, D.W. Optimized LC/MS/MS Analysis of Morphine and Codeine in Poppy Seed and Evaluation of Their Fate during Food Processing as a Basis for Risk Analysis. *J. Agric. Food Chem.* **2006**, *54*, 5292–5298. [CrossRef]
8. Carlin, M.G.; Dean, J.R.; Ames, J.M. Opium Alkaloids in Harvested and Thermally Processed Poppy Seeds. *Front. Chem.* **2020**, *8*, 737. [CrossRef]
9. Meos, A.; Saks, L.; Raal, A. Content of alkaloids in ornamental *Papaver somniferum* L. cultivars growing in Estonia. *Proc. Estonian Acad. Sci.* **2017**, *66*, 34. [CrossRef]
10. Cassella, G.; Wu, A.H.B.; Shaw, B.R.; Hill, D.W. The Analysis of Thebaine in Urine for the Detection of Poppy Seed Consumption. *J. Anal. Toxicol.* **1997**, *21*, 376–383. [CrossRef]
11. Hayes, L.W.; Krasselt, W.G. Concentrations of Morphine and Codeine in Serum and Urine after Ingestion of Poppy Seeds. *Clin. Chem.* **1987**, *33*, 806–809. [CrossRef] [PubMed]
12. Van Thuyne, W.; van Eenoo, P.; Delbeke, F.T. Urinary concentrations of morphine after the administration of herbal teas containing Papaveris fructus in relation to doping analysis. *J. Chromatogr. B* **2003**, *785*, 245–251. [CrossRef]
13. Thevis, M.; Opfermann, G.; Schänzer, W. Urinary Concentrations of Morphine and Codeine After Consumption of Poppy Seeds. *J. Anal. Toxicol.* **2003**, *27*, 53–56. [CrossRef]
14. Struempler, R.E. Excretion of Codeine and Morphine Following Ingestion of Poppy Seeds. *J. Anal. Toxicol.* **1987**, *11*, 97–99. [CrossRef] [PubMed]
15. Meadway, C.; George, S.; Braithwaite, R. Opiate concentrations following the ingestion of poppy seed products—evidence for 'the poppy seed defence'. *Forensic Sci. Int.* **1998**, *96*, 29–38. [CrossRef]
16. Braye, K.; Harwood, T.; Inder, R.; Beasley, R.; Robinson, G. Poppy seed tea and opiate abuse in New Zealand. *Drug Alcohol Rev.* **2007**, *26*, 215–219. [CrossRef]
17. EFSA. Update of the Scientific Opinion on opium alkaloids in poppy seeds. *EFS2* **2018**, *16*, 5243. [CrossRef]
18. Lachenmeier, D.W.; Sproll, C.; Musshoff, F. Poppy Seed Foods and Opiate Drug Testing-Where Are We Today? *Ther. Drug Monit.* **2010**, *32*, 11–18. [CrossRef]
19. Bishop-Freeman, S.C.; Fox, L.; Winecker, R.E.; Hudson, J.S. Death from Poppy Tea Consumption. *J. Anal. Toxicol.* **2020**, *44*, 734–740. [CrossRef]
20. King, M.A.; McDonough, M.A.; Drummer, O.H.; Berkovic, S.F. Poppy tea and the baker's first seizure. *Lancet* **1997**, *350*, 716. [CrossRef] [PubMed]
21. Spyres, M.B.; van Wijk, X.M.R.; Lapoint, J.; Levine, M. Two cases of severe opiate toxicity after ingestion of poppy seed tea. *Toxicol. Commun.* **2018**, *2*, 102–104. [CrossRef]
22. Garcia, M.R.; Swortwood, M.J.; Aune, C.N.; Ahmad, K.A. Maternal Poppy Seed Tea Ingestion and Ensuing Neonatal Abstinence Syndrome. *Neonatology* **2020**, *117*, 529–531. [CrossRef]
23. Commission Regulation. Commission Regulation (EU) 2021/2142 of 3 December 2021 amending Regulation (EC) No 1881/2006 as Regards Maximum Levels of Opium Alkaloids in Certain Foodstuffs. *Off. J. Eur. Union* **2021**, *L 433/8*.
24. Casado-Hidalgo, G.; Morante-Zarcero, S.; Pérez-Quintanilla, D.; Sierra, I. Pulsed ultrasound-assisted extraction followed by purification with SBA-15 for the control of opium alkaloids in biscuits and sponge cakes. *Microchem. J.* **2022**, *183*, 108059. [CrossRef]
25. Casado, N.; Gañán, J.; Morante-Zarcero, S.; Sierra, I. New Advanced Materials and Sorbent-Based Microextraction Techniques as Strategies in Sample Preparation to Improve the Determination of Natural Toxins in Food Samples. *Molecules* **2020**, *25*, 702. [CrossRef]
26. Pereira, J.A.M.; Gonçalves, J.; Porto-Figueira, P.; Figueira, J.A.; Alves, V.; Perestrelo, R.; Medina, S.; Câmara, J.S. Current trends on microextraction by packed sorbent - fundamentals, application fields, innovative improvements and future applications. *Analyst* **2019**, *144*, 5048–5074. [CrossRef] [PubMed]
27. Pereira, J.A.M.; Casado, N.; Porto-Figueira, P.; Câmara, J.S. The Potential of Microextraction Techniques for the Analysis of Bioactive Compounds in Food. *Front. Nutr.* **2022**, *9*, 825519. [CrossRef] [PubMed]
28. Casado, N.; Perestrelo, R.; Silva, C.L.; Sierra, I.; Câmara, J.S. Comparison of high-throughput microextraction techniques, MEPS and μ-SPEed, for the determination of polyphenols in baby food by ultrahigh pressure liquid chromatography. *Food Chem.* **2019**, *292*, 14–23. [CrossRef]
29. Casado, N.; Fernández-Pintor, B.; Morante-Zarcero, S.; Sierra, I. Quick and Green Microextraction of Pyrrolizidine Alkaloids from Infusions of Mallow, Calendula, and Hibiscus Flowers Using Ultrahigh-Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry Analysis. *J. Agric. Food Chem.* **2022**, *70*, 7826–7841. [CrossRef]
30. González-Gómez, L.; Pereira, J.A.M.; Morante-Zarcero, S.; Câmara, J.S.; Sierra, I. Green extraction approach based on μSPEed®followed by HPLC-MS/MS for the determination of atropine and scopolamine in tea and herbal tea infusions. *Food Chem.* **2022**, *394*, 133512. [CrossRef]
31. Casado-Hidalgo, G.; Morante-Zarcero, S.; Pérez-Quintanilla, D.; Sierra, I. Opium alkaloids in food products: Current and future perspectives. *Trends Food Sci. Technol.* **2021**, *108*, 92–102. [CrossRef]
32. Barea-Sepúlveda, M.; Duarte, H.; Aliaño-González, M.J.; Romano, A.; Medronho, B. Total Ion Chromatogram and Total Ion Mass Spectrum as Alternative Tools for Detection and Discrimination (A Review). *Chemosensors* **2022**, *10*, 465. [CrossRef]

33. European Commission. Commission Recommendation 2014/662/EU of 10 September 2014 on good practices to prevent and to reduce the presence of opium alkaloids in poppy seeds and poppy seed products. *Off. J. Eur. Union* **2014**, *L 271/96*. [CrossRef]
34. *ISO 3103*; Tea—Preparation of Liquor for use in Sensory Tests. ISO: Geneva, Switzerland, 2019.
35. European Union (2021). *SANTE/11312/2021. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*; Accredia: Rome, Italy; pp. 1–57. Available online: https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE_11312_2021.pdf (accessed on 18 July 2022).
36. European Commission Regulation No. 401/2006 of 23 February 2006 Laying Down the Methods of Sampling and Analysis for the Official Control of the Levels of Mycotoxins in Foodstuffs. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R0401&from=EN (accessed on 18 July 2022).
37. Tietje, C.; Brouder, A. Q2(R1) ICH guidelines (Internation Council for Harmonisation, 2005). In *Handbook of Transnational Economic Governance Regimes*; Brill: Leiden, The Netherlands, 2010; pp. 1041–1053. [CrossRef]
38. Vera-Baquero, F.L.; Morante-Zarcero, S.; Sierra, I. Evaluation of thermal degradation of tropane and opium alkaloids in gluten-free corn breadsticks samples contaminated with stramonium seeds and baked with poppy seeds under different conditions. *Foods* **2022**, *11*, 2196. [CrossRef] [PubMed]
39. Shetge, S.A.; Dzakovich, M.P.; Cooperstone, J.L.; Kleinmeier, D.; Redan, B.W. Concentrations of the Opium Alkaloids Morphine, Codeine, and Thebaine in Poppy Seeds are Reduced after Thermal and Washing Treatments but are Not Affected when Incorporated in a Model Baked Product. *J. Agric. Food Chem.* **2020**, *68*, 5241–5248. [CrossRef]